

DESCRIPTION

Source *E. coli*-derived human PSAT1 protein
Met1-Leu370
Accession # Q9Y617-1
with a C-terminal 6-His tag

N-terminal Sequence Analysis Met1

Predicted Molecular Mass 41 kDa

SPECIFICATIONS

SDS-PAGE 40 kDa, under reducing conditions

Activity Measured by its ability to produce 3-phosphooxypyruvate.
The specific activity is >130 pmol/min/μg, as measured under the described conditions.

Endotoxin Level <0.10 EU per 1 μg of the protein by the LAL method.

Purity >95%, by SDS-PAGE visualized with Silver Staining and quantitative densitometry by Coomassie® Blue Staining.

Formulation Supplied as a 0.2 μm filtered solution in MES and NaCl. See Certificate of Analysis for details.

Activity Assay Protocol

- Materials**
- Assay Buffer: 50 mM Tris, 16 mM Ammonium Acetate, pH 8.5
 - Recombinant Human PSAT1 (rhPSAT1) (Catalog # 10342-PS)
 - Recombinant Human PHGDH (rhPHGDH) (Catalog # 10131-DH)
 - O-Phospho-L-serine (Tocris, (Catalog # 0238) 100 mM stock in 20 mM Tris, pH 8.5
 - β-Nicotinamide Adenine Dinucleotide, reduced (NADH) (Sigma, Catalog # N8129), 20 mM stock in 0.1 M Sodium Borate, pH 9.0
 - α-Ketoglutaric acid (Sigma, Catalog # K2010), 1 M stock in deionized water
 - Pyridoxal 5'-phosphate (Sigma, Catalog # P9255), 100 mM stock in 1 M HEPES, pH 8.0
 - 96-well Clear Plate (Catalog # DY990)
 - Plate Reader (Model: SpectraMax Plus by Molecular Devices) or equivalent

- Assay**
1. Prepare a substrate mixture containing 0.4 mM NADH, 2 mM α-Ketoglutaric acid, 40 μM Pyridoxal 5'-phosphate, 2 mM O-Phospho-L-serine and 14 μg/mL rhPHGDH in Assay Buffer. Mix well and incubate at room temperature for 5 minutes.
 2. Dilute rhPSAT-1 to 20 μg/mL in Assay Buffer.
 3. Load 50 μL of 20 μg/mL rhPSAT-1 into wells of a plate, and start the reaction by adding 50 μL of substrate mixture. Include a Substrate Blank containing 50 μL Assay Buffer and 50 μL of substrate mixture.
 4. Read plate at an absorbance of 340 nm in kinetic mode for 10 minutes with a lag time of 3 minutes.
 5. Calculate specific activity:

$$\text{Specific Activity (pmol/min/}\mu\text{g)} = \frac{\text{Adjusted } V_{\text{max}}^* (\text{OD/min}) \times \text{well volume (L)} \times 10^{12} \text{ pmol/mol} \times (-1)}{\text{ext. coeff}^{**} (\text{M}^{-1}\text{cm}^{-1}) \times \text{path corr.}^{***} (\text{cm}) \times \text{amount of enzyme } (\mu\text{g})}$$

*Adjusted for Substrate Blank

**Using the extinction coefficient 6220 M⁻¹cm⁻¹

***Using the path correction 0.32 cm

Note: the output of many spectrophotometers is in mOD

Final Assay Conditions

- Per Well:
- rhPSAT-1: 1.0 μg
 - O-Phospho-L-serine: 1 mM
 - NADH: 0.2 mM
 - α-Ketoglutaric acid: 1 mM
 - Pyridoxal 5'-phosphate: 20 μM
 - rhPHGDH: 0.7 μg

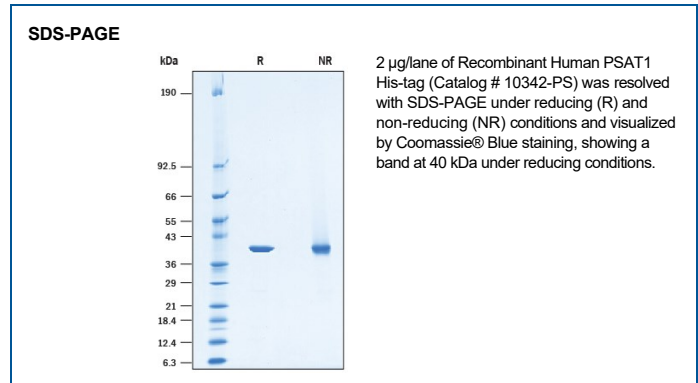
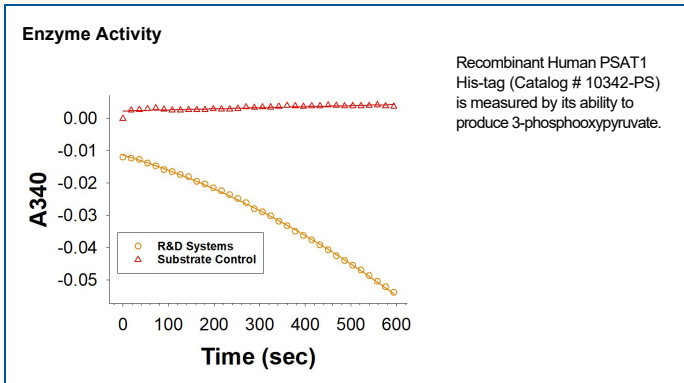
PREPARATION AND STORAGE

Shipping The product is shipped with polar packs. Upon receipt, store it immediately at the temperature recommended below.

Stability & Storage Use a manual defrost freezer and avoid repeated freeze-thaw cycles.

- 6 months from date of receipt, -20 to -70 °C as supplied.
- 3 months, -20 to -70 °C under sterile conditions after opening.

DATA



BACKGROUND

Phosphoserine aminotransferase (PSAT1), a pyridoxal-phosphate (PLP)-dependent cytosolic protein catalyzes the conversion of 3-phosphohydroxyypyruvate into phosphoserine; the reversible second step in the de novo serine synthesis pathway. PSAT1 forms an active a/b domain structure homodimer where each monomer is 40 kDa and 370 amino acids (1). PSAT1 contains a catalytic lysine and bound PLP in the active site. Although serine is considered a nonessential amino acid, de novo synthesis is essential in the brain due to insufficient delivery through the blood brain barrier. PSAT1 expression is concentrated in brain, liver, kidney, and pancreas suggesting these tissues are primary sites for de novo serine synthesis (2). Mutations in PSAT1 can cause Neu-Laxova syndrome, a serine-deficiency disorder (3). PSAT1 has been reported to signal through the GSK3beta/beta-catenin pathway (4, 5), regulate alpha keto-glutarate in a role essential for embryonic stem cell self-renewal and pluripotency (6) and promote migratory metastasis and proliferation in roles independent of serine synthesis (7,8). PSAT1 is up-regulated and associated with poor prognosis in several cancers including nasopharyngeal (9), breast (10), and esophageal squamous carcinoma (11). PSAT1 has been proposed as a promising target for tumor suppression in colorectal, esophageal, and breast cancers (8, 12, 13). It has been proposed as a target in brain tumors through a novel stabilization mechanism (14) and for inhibition of the serine production pathway in tuberculosis (15).

References:

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